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LARVA RELEASE IN RESPONSE TO LIGHT BY THE COMPOUND ASCIDIANS *DISTAPLIA OCCIDENTALIS* AND *METANDROCARPA TAYLORI*

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Light has been implicated as a trigger to gamete release by several solitary ascidians (see Lambert and Brandt, 1967, for review). Most solitary ascidians are oviparous with large numbers of relatively small eggs which develop rapidly into a simplified tadpole larva which swims for a short time before selecting a suitable substrate and metamorphosing (Berrill, 1950). Compound ascidians, on the other hand, generally produce only a few large eggs which develop ovoviviparously into a highly differentiated tadpole larva that is only released when development of the swimming larva is complete. Many observations of larva release by compound ascidians have been reported, which include members of both orders in which brooding is common (Abbott, 1955; Costello, Davidson, Eggers, Fox and Henley, 1957; Grave, 1936, 1937; Grave and Woodridge, 1924; Oka, 1943; Scott, 1954). *Aplidium* (= *Amaroucium*) *constellatum* (Scott, 1954; Costello, Davidson, Eggers, Fox and Henley, 1957), *Perophora viridis* (Costello *et al.*, 1957), *Polyandrocarpa tincta* (Grave, 1936) and *Botryllus schlosseri* (Grave and Woodbridge, 1924; Grave, 1937) seem to release tadpoles during the morning when

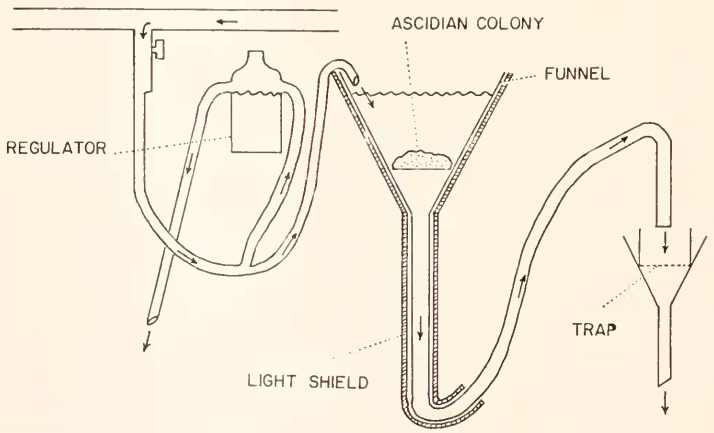

FIGURE 1. Diagram of the tunicate tadpole collector. The water level is maintained by the heights of the regulator and second outflow u tube. The larvae are collected on 370μ Nytex mesh in the trap.

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subjected to natural illumination while *Polycitor mutabilis* (Oka, 1943), *Metandrocarpa taylori* (Abbott, 1955) and *Symplegma viride* (Grave, 1937) have been reported to release larvae throughout a normal day-night cycle. In most of these studies the primary interest was embryological or behavioral or concerned with metamorphosis; larva release was an incidental observation.

In the present study we have examined in some detail larva release by two compound ascidians, *Distaplia occidentalis* (Aplousobranchiata) and *Metandrocarpa taylori* (Stolidobranchiata), in two light regimes. Larva release under natural illumination has been studied in detail over long periods of time throughout the 24 hour cycle. Experimental light-dark cycles have allowed us to clarify the role of light in the natural release cycle of these two ascidians. A portion of the results reported here were presented in a preliminary form elsewhere (Watanabe and Lambert, 1971). The observations and experiments were undertaken at the Friday Harbor Laboratories during 1968 and 1969.

Materials and Methods

Experimental animals

Large colonies of *Distaplia occidentalis* were collected from logs in Jakle's Lagoon on the east side of San Juan Island, San Juan County, Washington. Colonies of *Metandrocarpa taylori* were obtained by dredging ascidian-encrusted cobbles from Peavine Pass between San Juan and Orcas Islands. The colonies of *Metandrocarpa* are broadly attached to the substratum so that it was necessary to leave the colonies attached to their rocks or shells during all laboratory observations and manipulations. Colonies of *Distaplia* are rather pedunculate, making it possible to remove entire colonies without apparent damage. The animals were maintained on running sea water tables until use (generally 2–3 days). Because the Friday Harbor Laboratories sea water system is non-filtered and non-recirculating, filter feeders such as ascidians flourish and grow for long periods of time.

Experimental apparatus and methods

Larva release under various conditions of illumination was examined by means of the larva collector shown in Figure 1. Essentially this is an inverted version of the tunicate egg collector used by Huus (1939). Running sea water constantly swirls through the 254 cm diameter funnel, the water level being regulated by the input flow rate and the heights of the outflow u-tube and regulator. Immediately after release the larvae are washed through the outflow tube to the filter trap which is constructed of a 9 cm diameter circle of 370 μ pore size Nytex cloth cemented to a short Plexiglas cylinder. The 370 μ mesh size filter was chosen because it is large enough to pass diatoms, small algae, *etc.*, without becoming clogged, but small enough to retain the larvae of these ascidians. The larva collector was covered with black plastic and aluminum foil to exclude light except from above. For studies on larva release in the dark the top of the funnel was also light proofed. The illumination for laboratory studies on larva release in the light was furnished by the general fluorescent lighting of the laboratory with an additional 40 watt incandescent lamp 30 cm above the funnel. Experiments involving natural illumination were undertaken with the larva collector installed outside the laboratory away

from all extraneous artificial illumination. The water temperature varied from 11°C to 15°C during the course of these studies. For any experimental series, however, the water temperature was constant within 1°C.

Results

Distaplia occidentalis

The breeding season of *Distaplia* extends from early April to late August with a maximum from May to July. Most of our observations on this ascidian took place between mid-May and mid-June. Larva release by several colonies was examined with similar results so we will present the data obtained from a single representative colony. This colony was 6.5 cm long, 6.0 cm wide and 3 cm in

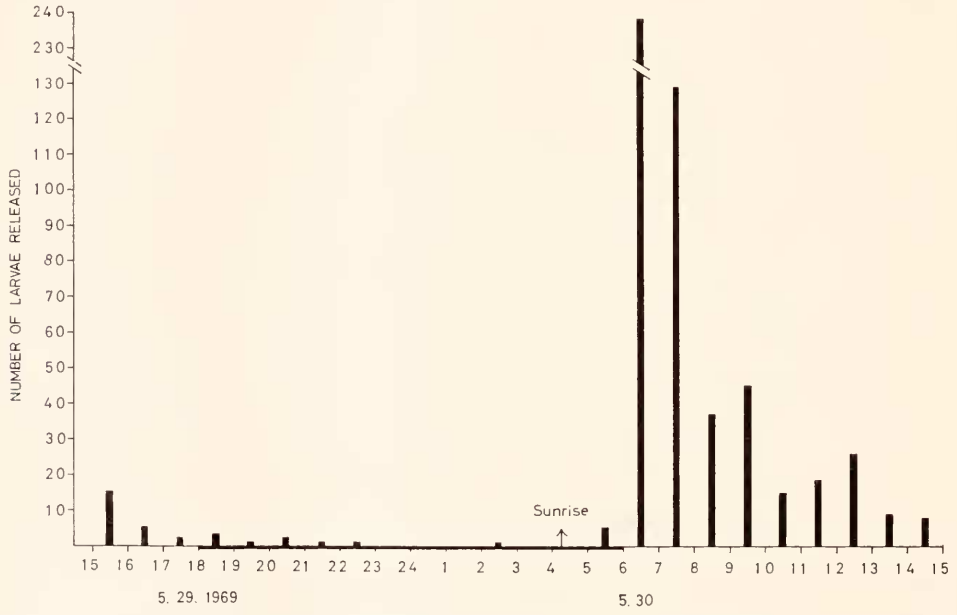

Figure 2. Larva release under conditions of natural illumination by *Distaplia occidentalis*.

height. The colony contained about 150 systems, each composed of 12–15 adult zooids.

The first series of observations examined larva release under natural illumination. During 24 hours of natural illumination a total of 563 larvae were released. As shown in Figure 2, most of the larvae were liberated during the morning hours with 483 (85.8%) being released before noon, 65 (11.5%) released between 1200–1800 and only 15 (2.7%) released during the night (1800–0600). A longer term experiment is shown in Figure 3. Here we have followed larva release during the night, morning and afternoon of three successive days with the same morning peak of larva release being evident. Seventy-five per cent of the 1838 larvae were

released during the morning hours. All of these larvae were fully mature; *Distaplia* does not release eggs or partially developed embryos.

Larva release under natural illumination suggests that light following a dark period elicits the release of larvae. To investigate further this possibility a series of experiments were conducted in which we artificially controlled the timing and duration of the dark and light periods. Figure 4 shows larva release during 44 hours of darkness followed by 3 hours of light, after which the colony was again darkened. During the initial dark period only 20 larvae were released or 5 per hour (Z/h = .5) on the average. Immediately after return to light, larva release began with a maximum within one hour. During the 3 hours of illumination 390 larvae were released (Z/h = 130). Upon returning the colony to darkness for an additional 15 hours, only 7 larvae (Z/h = .6) were released. Under our conditions at least 15 minutes of illumination are required for larva release even though

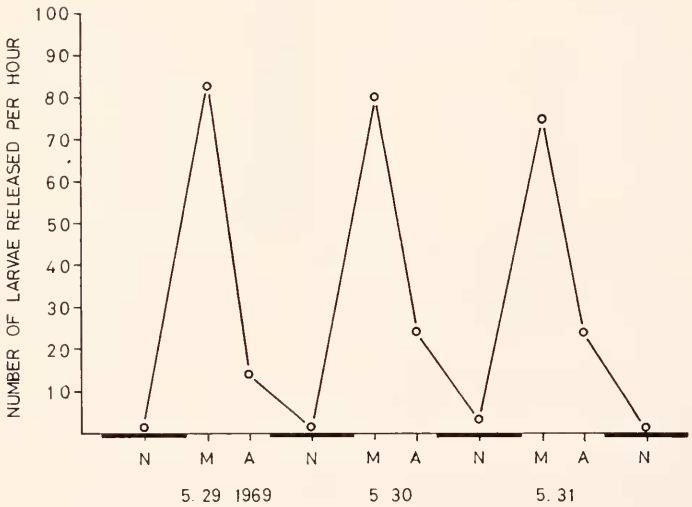

FIGURE 3. Larva release during 3 successive days of natural illumination by *Distaplia occidentalis*. The abbreviations indicate: U = 1800–0600, M = 0600–1200, A = 1200–1800

the larvae were not released until after return to darkness. We then examined the relationship between the duration of darkness and the number of larvae released upon return to light. Table I shows that there is a clear tendency for more larvae to be released after a longer period of darkness than a shorter one regardless of the time of day.

During long periods of continuous darkness very few larvae are released (Table II). We would have predicted that under conditions of continuous illumination, after the initial swarm of larvae were released the rate of release would fall to that of the colony under continuous darkness if a dark period is absolutely requisite for release. That this is not the case is shown in Table II. Here it can be seen that after the initial large release the level of release remains higher than the dark-adapted colony. It should be noted, however, that the initial rate of release during

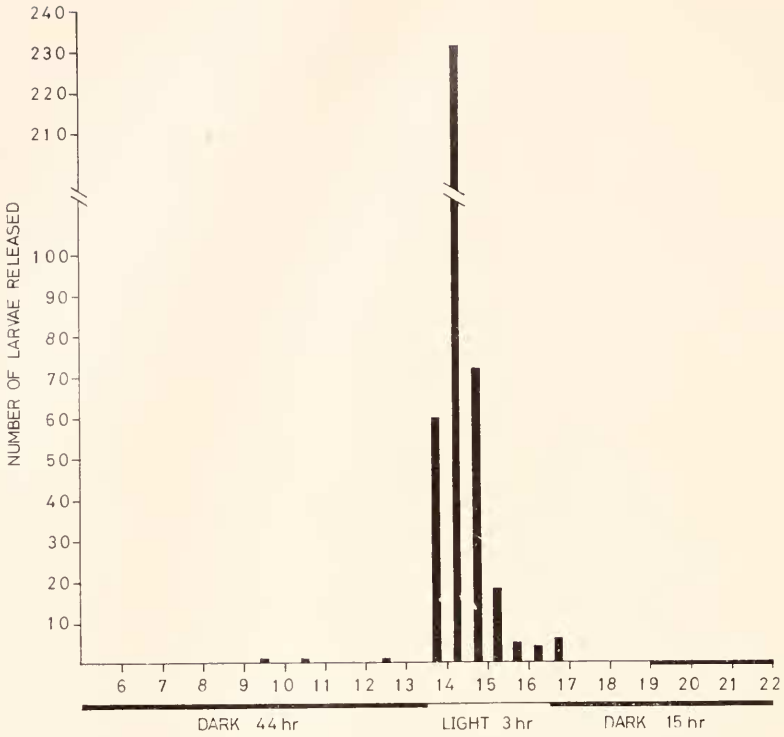

FIGURE 4. The effect of light on larva release by *Distaplia occidentalis*.

the first 6 hours following darkness was 203.5 Z/h as compared to the long term average rate of 46.1 Z/h.

Metandrocarpa taylori

Metandrocarpa breeds throughout the year in Washington waters, which is consistant with its breeding cycle in California (Haven, 1971) and the reproduction

TABLE I

The relationship between the duration of the dark period and number of larvae released on return to light by Distaplia occidentalis

Duration of dark period	Duration of light period	No. of larvae released	z/h
15 hr →	3 hr (19:00–22:00)	146	48.7
20.5 hr	3 hr (14:30–17:30)	173	57.7
42.5 hr	0.5 hr (13:30–14:00)	144	57.6
44 hr	3 hr (13:30–16:30)	390	130.0
48 hr	4 hr (15:00–19:00)	1131	188.5
63 hr	5 hr (08:30–13:30)	520	104.0
84.5 hr	6 hr (08:00–14:00)	1554	310.8

TABLE II

Summary of larva release by Distaplia occidentalis *under all experimental light regimes. D → L refers to larva release occurring up to six hours after the onset of illumination*

Illumination	Duration	No. of larvae released	z/h
Dark	350 hr	332	0.9
Light	238.75 hr	11008	46.1
D → L	30.75 hr	6258	203.5
	Total 588.75 hr	11340	

of *Metandrocarpa uedai* in Japanese waters (Watanabe and Tokioka, 1972). Our representative colony of *Metandrocarpa* consisted of 580 blastozooids above 4.0 mm along the antero-posterior axis.

Larva release under a natural light regime roughly parallels the results with *Distaplia*. During 24 hours of hourly observations (Fig. 5), 82 larvae were released. The number of larvae released began to increase around 0700 hours, reached a peak between 0900 hours and 1000 hours and declined afterwards; only a few were randomly released during the rest of the day. The same colony was observed during 7 successive days of natural illumination (Fig. 6). Here we see repeated the early morning mass release of larvae that was observed during the single diurnal cycle. Table III summarizes the long-term observations. Unlike *Distaplia*, *Metandrocarpa* often released a few immature tadpoles and unhatched embryos along with the actively swimming tadpoles. The proportion of immature stages released is the lowest during the peak morning swarms with the afternoon and night being about equal.

The observations under the normal day-night regime again suggested photically controlled larva release; therefore we turned our attention to experimental modifications of the duration and timing of illumination. These experiments were undertaken with several large colonies of *Metandrocarpa* with perfectly uniform results. Here we report on larva release by a representative colony which contained 350 adult zooids above 4.0 mm along the antero-posterior axis. Figure 7 shows the

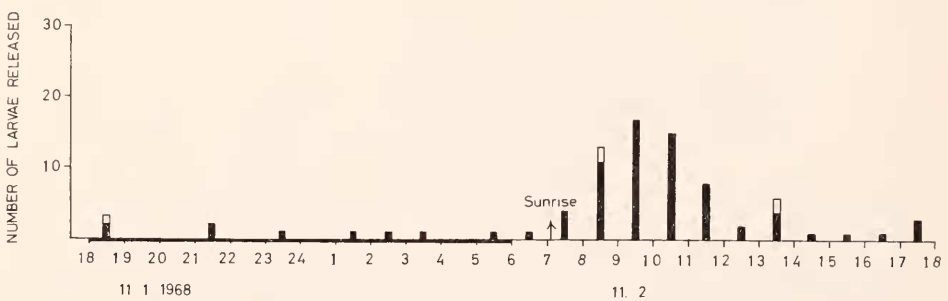

FIGURE 5. Release of larvae under natural illumination by *Metandrocarpa taylori*. The blackened bars indicate the number of mature larvae expelled, the white portions indicate the number of immature stages.

TABLE III

Larva release by Metandrocarpa taylori *under natural illumination. The immature tadpole category includes all developmental stages incapable of effective locomotion*

Illumination	Duration	Mature tadpoles	Immature tadpoles	Total	z, h
Night	84 hr	34 (76.6%)	11 (24.4%)	45	0.5
Day (07:00–12:00)	35 hr	247 (93.6%)	17 (6.4%)	264	7.5
Day (12:00–18:00)	49 hr	39 (70.9%)	16 (29.1%)	55	1.1
	Total 168 hr	320 (87.9%)	44 (12.1%)	364	2.2

results of holding the colony under continuous darkness for 43 hours, then exposing it to continuous light. Again, we see the massive larva release upon returning the colony to light. The number of larvae released on return to light seems to be related to the duration of the dark period (Table IV) with a clear tendency to release fewer immature stages after the longest dark periods. We then determined the minimal duration of exposure to light that would elicit larva release after a suitable dark period. Again, 15 minutes of exposure to light seems necessary for release even though some of the tadpoles were released after return to darkness. Larva release during long, continuous exposure to light or darkness is shown in Table V. Small numbers of larvae were irregularly released during both treatments. Again more larvae were released under illumination than darkness. More immature tadpoles were released during continuous light or dark than under natural or experimental dark-light cycles.

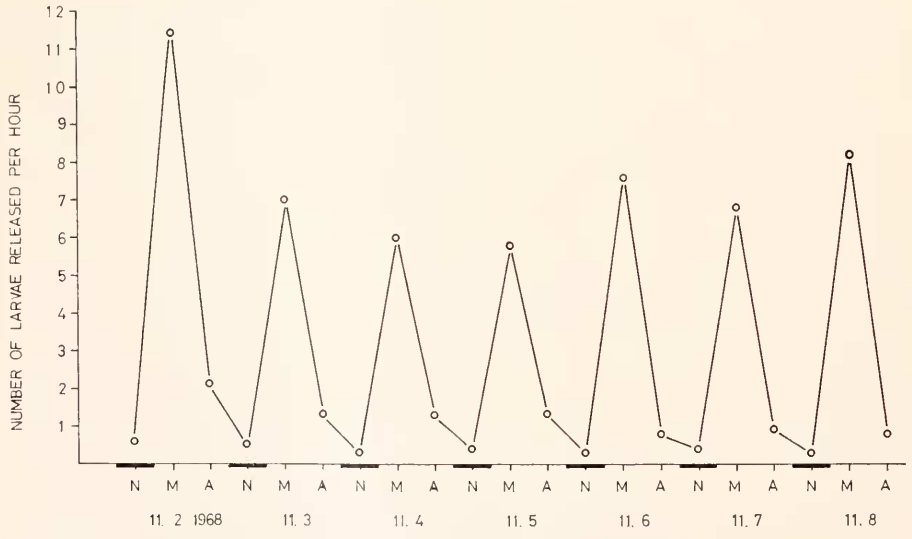

FIGURE 6. Larva release during 7 successive days of natural illumination by *Metandrocarpa taylori*. The abbreviations are the same as in Figure 3.

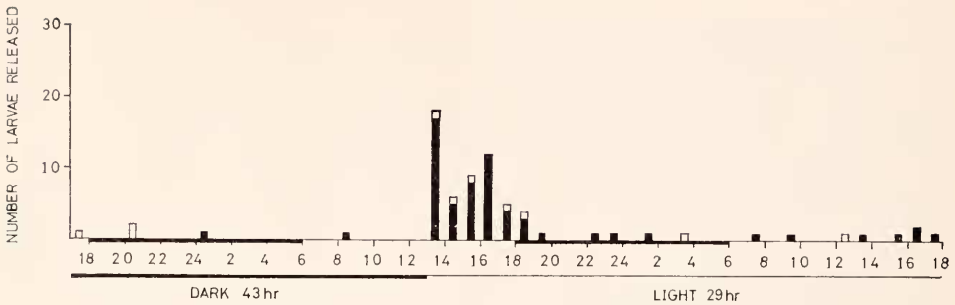

FIGURE 7. The effect of light on larva release by *Metandrocarpa taylori.*

DISCUSSION

Our observations clearly indicate that both *Metandrocarpa taylori* and *Distaplia occidentalis* preferentially release their tadpole larvae upon exposure to light after a period of darkness. This generalization holds for both larva release under a natural day-night cycle and under experimentally altered periods of darkness and illumination. These findings are similar to most of the reports on larva release by compound ascidians. *Botryllus schlosseri* (Grave and Woodridge, 1924; Grave, 1937), *Polyandrocarpa tincta* (Grave, 1936) and *Perophora viridis* (Costello *et al.*, 1957) all release their larvae during the morning in the laboratory when the laboratory was darkened at night. *Aplidium* (= *Amaroucium*) *constellatum* (Scott, 1954; Costello *et al.*, 1957) normally releases larvae 20 minutes after dawn, but can be induced to release 20 minutes after the onset of illumination if maintained in the dark until later in the day. Two other ascidians, *Polycitor mutabilis* (Oka, 1943) and *Symplegma viride* (Grave, 1937) apparently release larvae sporadically throughout a diurnal light cycle. Earlier work with *Metandrocarpa taylori* (Abbott, 1955) also suggested that larva release by this ascidian might not be under photic control. Abbott (1955) observed sporadic release of larvae throughout a day-night cycle by colonies held in finger bowls of still sea water, in the presence of very dim light during the night. It is apparent that Abbott's observations are not directly comparable with ours, because of the different conditions under which release was studied. It would be of considerable interest to examine larva release

TABLE IV

The relationship between duration of the dark period and the number of larvae released on return to light by Metandrocarpa taylori

Duration of dark period	Duration of light period	Mature tadpoles	Immature tadpoles	Total	z/h
13 hr →	5 hr (07:00–12:00)	4	1	5	1.0
20 hr	4 hr (14:00–18:00)	16	3	19	4.8
40 hr	5 hr (07:00–12:00)	22	3	25	5.0
43 hr	5 hr (13:00–18:00)	46	4	50	10.0
70 hr	3 hr (19:00–22:00)	42	0	42	14.0
90 hr	5 hr (09:00–14:00)	74	0	74	14.8
96 hr	4 hr (15:00–19:00)	100	0	100	25.0

by *Symplegma viride* and *Polycitor mutabilis* for long periods in our larva collector to see if these ascidians would also exhibit photic control in running sea water.

Although we did not specifically study the mechanism of larva release, certain aspects of the morphological basis of brooding in *Metandrocarpa* (Abbott, 1955) and *Distaplia* (Berrill, 1948) have been described which are important in any consideration of the mechanism of larva release. The development of *Distaplia* embryos occurs within a brood pouch which is essentially the distal portion of the oviduct (Berrill, 1948). One finds a complete series of developmental stages within the sac with fully formed tadpoles at the distal and early cleavage stages at the proximal end of the sac. As the parental zooid regresses the brood pouch can become completely isolated from the parental zooid leaving the intact brood sac as an independent structure with its contained embryos as the structure from which the larvae are released. Thus larva release is not likely to be under control of the parental nervous system in this species. Reese (1967) has shown that the isolated gonoducts of *Ciona intestinalis* can be induced to spawn by exposure to light which opens the possibility that the brood sac itself may be responding to light in *Distaplia*. Light induced larval activity is probably not a factor in larva release by *Distaplia* as the

TABLE V

The effect of continuous darkness and continuous illumination on larva release by Metandrocarpa taylori

Duration of dark or light period	Mature tadpoles	Immature tadpoles	Total	z/h
136 hr (dark)	13 (68.4%)	6 (31.6%)	19	0.1
115 hr (light)	94 (77.7%)	27 (22.3%)	121	1.1

larvae are not active at the time of actual escape from the colony (Ritter and Forsyth, 1917). In contrast to *Distaplia*, *Metandrocarpa* broods its embryos in the peribranchial cavity, about 15–20 embryos of various stages being found in a large individual. Our findings that 12% of the young of *Metandrocarpa* are released in an immature state are in agreement with those of Abbott (1955), who suggested that the immature larvae and embryos might be accidentally released along with the release of faeces. That the proportion of immature embryos was at a minimum during the morning when the greatest number of larvae are released under natural light conditions (Table III) suggests that the release of mature larvae and immature embryos are under fundamentally different controls. Perhaps the mature, swimming larvae are triggered into swimming activity by light following darkness, while the immature stages are released by mild random contractions of the parent. This hypothesis gains support from the finding that roughly similar numbers of immature tadpoles were released under all conditions of illumination (Table III) while the number of mature tadpoles released showed a nearly tenfold increase following exposure to light. *Metandrocarpa uedai* (Watanabe and Tokioka, 1972), in contrast to *M. taylori*, releases only mature larvae, which suggests that this species has evolved a mechanism for the retention of immature stages that is lacking in *M. taylori*.

The ecological significance of ascidians releasing the majority of their larvae during the few hours following dawn is problematical. Possibly the hour of release is not crucial, the important factor being a large number of larvae released at about the same time. It is difficult to see how synchronous larva release would make any adaptive difference whatsoever in the reproductive success of these ascidians (see Millar, 1972 for review of reproductive strategy). A more plausible explanation, we feel, is related to the function of the ascidian tadpole: habitat selection (Berrill, 1950). Differential orientations to light are important in habitat selection by ascidian larvae. Initially the larvae are photo-positive; later they become photo-negative. Only during the photo-negative period do the larva metamorphose, chiefly in shaded portions of rocks, logs or algae. Thus, it is important that each larva should have a maximum period for seeking a suitable substratum during the day of release. Presumably those larvae which do not find an adequately shaded spot before nightfall would tend to stand a poor chance for colony development and reproductive success.

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Summary

1. Larva release under natural and artificially determined light regimes was studied in the colonial ascidians *Distaplia occidentalis* and *Metandrocarpa taylori*.

2. Under natural illumination, both species show a clear tendency to release their larvae during the morning hours. Sunrise marks the beginning of larva release.

3. Under experimental light conditions, larva release can be initiated at any time of the day or night by exposing suitably dark conditioned colonies to light. Transfer from light to darkness has no effect on larva release.

4. The number of larvae released upon exposure to light is related to the duration of the dark period. A greater number of larvae are released after a long dark period than a short one.

5. The minimum duration of exposure to light that will elicit larva release by dark adapted colonies is 15 minutes. Larva release continues after return to darkness, providing the duration of illumination is adequate.

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